Running head: Parasites in trophic cascades

An experimental test of how parasites of predators can influence trophic cascades and ecosystem functioning

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Abstract

Parasites can shape the structure and function of ecosystems by influencing both the density and traits of their hosts. Such changes in ecosystems are particularly likely when the host is a predator that mediates the dynamics of trophic cascades. Here, we experimentally tested how parasite load of a small predatory fish, the threespine stickleback, can affect the occurrence and strength of trophic cascades and ecosystem functioning. In a factorial mesocosm experiment, we manipulated the density of stickleback (low vs. high), and the level of parasite load (natural vs. reduced). In addition, we used two stickleback populations from different lineages: an Eastern European lineage with a more pelagic phenotype (Lake Constance) and a Western European lineage with a more benthic phenotype (Lake Geneva). We found that stickleback caused trophic cascades in the pelagic but not the benthic food chain. Evidence for pelagic trophic cascades was stronger in treatments where parasite load of stickleback was reduced with an antihelmintic medication, and where fish originated from Lake Constance (i.e. the more pelagic lineage). A structural equation model revealed that differences in stickleback lineage and parasite load were most likely to impact trophic cascades via changes in the composition, rather than overall biomass, of zooplankton communities. Overall, our results provide experimental evidence that parasites of predators can influence the cascading effects of fish on lower trophic levels with consequences on ecosystem functioning.

Keywords: parasites, density-mediated interactions, trait-mediated interactions, trophic cascades, stickleback, mesocosms, structural equation models, ecosystem functioning.

Introduction

In a classic trophic cascade, predators alter the biomass and/or community structure (species biomass and composition) of lower trophic levels and even ecosystem functioning (e.g. gross primary productivity and decomposition) via a combination of direct and indirect ecological effects (Ripple et al., 2016; Polis et al., 2000). In food chains composed of predators, grazers, and primary producers, predators can increase the biomass of primary producers by decreasing the density or biomass of grazers (density-mediated indirect effects or DMIE, Fig. 1a) (Abrams, 1995). Alternatively, predators can change the behavior of grazers, for example by reducing their activity and feeding rates, and thereby increase the biomass of primary producers via trait-mediated indirect effects (TMIE Fig. 1b) (Abrams, 1995, 2007). In addition, predators might shift the composition of grazer communities and thereby affect primary producers (Schmitz, 2006).

Previous work on trophic cascades has shown that intra-specific variation morphology, activity levels, consumption rates, or hunting strategies can have profound effects on the occurrence and strength of trophic cascades (Start and Gilbert, 2017; Schmitz, 2008; Abrams, 2007; Borer et al., 2005; Schmitz, 2008; Post et al., 2008; Rudman et al., 2016), and many of these traits can be directly modified by parasite infections (Lafferty, 2006; Lafferty and Kuris, 2012; Hatcher et al., 2006). Parasites of predators can have trophic cascading effects both by reducing the population densities of their hosts (DMIE) and by changing host traits such as foraging activity and performance (TMIE, Fig. 1c), but the interaction of such parasite and predator effects have so far received little attention (Hatcher and Dunn, 2011; Lefèvre et al., 2009). Previous research in aquatic systems has tested how parasites of grazers (e.g. *Daphnia*) can mediate the strength of trophic cascades by changing grazer foraging behavior (Duffy, 2007; Wood and Johnson, 2015), but we still lack evidence on whether and how parasites of predators affect the occurrence and strength of trophic cascades.

Whether parasites impact trophic cascades by changing predator density or changing predator behavior, the magnitude of their impacts likely depends on the co-evolutionary history between parasites and their hosts (Eizaguirre et al., 2012) as well as the host trophic position (Lafferty, 2006). For instance, the evolution of host resistance to parasites can determine how

strongly parasites affect host population density (DMIE; Hudson et al., 1998), as well as host condition and behavior for a given density (Anaya-Rojas et al., 2016; Buck and Ripple, 2017). So far, studies have addressed how variation in ecological conditions (e.g., resources or temperature) affect the dynamics of host-parasite interactions (Duffy et al., 2012; Wolinska and King, 2009).

To test whether parasites can influence variation in predator-mediated effects on ecosystem functioning in general, and on trophic cascades in particular, we performed a mesocosm experiment where we manipulated the density and parasite load of threespine stickleback (*Gasterosteus aculeatus*). We were interested in addressing three main questions: (i) how do different parasite loads influence the direct and indirect ecological effects of stickleback?, (ii) what ecological pathways are most likely to underlie these effects?, and (iii) how do stickleback lineages vary in their response to parasite load in a way that might impact their ecosystem effects?

In natural populations, stickleback are host to multiple parasite species that can impact their behavior and therefore their trophic interactions (Barber, 2013). For instance, parasites such as *Apatemon* spp. and *Diplostomum* spp. can reduce stickleback consumption rates by reducing their vision (Seppälä et al., 2011). Intestinal parasites such as the cestode *Schistocephalus solidus* can actively manipulate stickleback feeding behavior (Milinski, 1984), whereas ecotoparasites such as *Gyrodactylus gasterostei* can indirectly affect stickleback feeding behavior (Anaya-Rojas et al., 2016) and reduce body condition (Eizaguirre et al., 2009). In a previous mesocosm experiment, experimental infections of stickleback with *Gyrodactylus gasterostei* reduced stickleback body condition, thereby changing the effect of stickleback on both community composition and ecosystem functioning (Brunner et al., 2017). However, natural populations of stickleback are exposed to a large diversity of parasites (Eizaguirre et al., 2011), resulting in different co-evolutionary dynamics across fish populations (Eizaguirre et al., 2012). Hence, instead of manipulating individual parasite species (Brunner et al., 2017), we experimentally reduced parasite load by medicating stickleback with an anti-helminth treatment. We used structural equation models to tease apart the direct and indirect effects of stickleback's parasite load and density on a broad range of ecosystem properties, including the total biomass and composition of the grazer community, the biomass of primary producers, and ecosystem

functioning measured using an index of ecosystem multi-functionality (EMF) (Byrnes et al., 2014).

The co-evolutionary history of stickleback populations with their parasite community might also influence how parasite load affects stickleback-mediated trophic cascades. In our experiment, we used stickleback from two European lineages that have recently colonized Switzerland: an Eastern European lineage, represented by the population in Lake Constance, and a Western European lineage, represented by the population in Lake Geneva (Lucek et al., 2010; Fang et al., 2018; Best et al., 2017). Previous work has shown that stickleback from the Lake Constance population have a more pelagic phenotype and feed more efficiently on pelagic prey than stickleback from the Lake Geneva population (Lucek et al., 2010; Best et al., 2017). In addition, these two populations have different parasite communities (Karvonen et al., 2015). We hypothesized that such lineage-specific differences might influence variation in the strength of stickleback-mediated trophic cascades in mesocosm ecosystems.

Materials and methods

Mesocosm experiment

We conducted a large-scale mesocosm experiment with 45 outdoor cattle tanks (1000 L each) from 12^{th} of May to 14^{th} of June 2014 at the Center for Ecology, Evolution and Biogeochemistry of Eawag (Swiss Federal Institute of Aquatic Science and Technology) in Kastanienbaum, Switzerland. We collected male sticklebacks at the shores of Lake Constance (47^{o}29'56.6" N, 9^{o}33'26.6"E) and Lake Geneva (46^{o}23'53 N, 6^{o}53'08.6"E) with minnow traps placed overnight the first week of April 2014. All fish were kept in aquaria and fed with chironomids until the start of the experiment. In a complete randomized block design (Fig. 2a), replicated five times, we crossed 8 factorial combinations of stickleback lineage (Constance or Geneva), two levels of fish densities (low= three fish or high= ten fish, LD and HD respectively), and parasite load (natural or reduced, $+I$ and $-I$ respectively), plus a control tank (no fish added). Stickleback densities used in this experiment are biologically relevant for this type of experiment (see Matthews et al., 2016; Best et al., 2017; Brunner et al., 2017). During the experiment, dead fish were replaced with fish of similar size and from the same treatment combination to keep fish density constant during the

experiment and avoid confounding effects of fish mortality.

Manipulating parasite load

Prior to the mesocosm experiment, we conducted a laboratory experiment to test the effectiveness of Praziquantel (Koi MED®Worm-Ex®, hereafter, Wormex) in reducing the parasite load of stickleback (36 fish in total: 18 from each lineage). Wormex is a commercial antihelmintic medication that reduces the prevalence of common fish parasites such as *Apatemon* spp., *Diplostomum* spp., *Gyrodactylus* spp., and *Schistocephalus solidus* and with minimal side effects on fish health and behavior (MacColl and Chapman, 2010). Fish assigned to the $-I$ treatment (reduction of parasite load) were treated with 10 mg/L of Wormex dissolved in a 0.64% NaCl solution, following the manufacturer's specifications. Fish assigned to the $+I$ treatment were exposed to the saline solution without Wormex. After a three-hour bath, all fish were transferred to clean tap water and kept in the laboratory for two weeks in non-flow aquaria. All individuals were completely dissected, visually inspected under an dissection scope (Leica MZ6) for the presence and abundance of four focal helminth parasites (*Apatemon* spp., *Diplostomum* spp., *Gyrodactylus* spp., and *Schistocephalus solidus*) and the protozoa, *Ichthyophthirius multifiliis*. All parasites were identified morphologically. For each individual, we screened the body surface and gills for *Gyrodactylus* spp. and *Ichthyophthirius multifiliis*, then we dissected their body cavity to check for *Schistocephalus solidus*. Eyes were removed and opened to check for *Diplostomum* spp. and *Apatemon* spp. cysts. This initial laboratory confirmed that Wormex treatment significantly reduced parasite load, mainly via reductions of *Gyrodactylus* spp. (see Table-S1 in Appendix S1), and significantly changed the parasite community structure (MLRT= 33.65, P= 0.002).

Following this laboratory experiment, we used a similar approach to manipulate the parasite load of fish used in the mesocosm experiment. Specifically, we exposed fish intended for the $-I$ treatment to 10 mg/L Wormex, and $+I$ fish to a saline solution without Wormex. We treated 130 fish (65:65, Constance:Geneva) in non-flow aquariums, and after three hours of treatment all fish were transferred to clean tap water and held for two weeks prior to their addition to the mesocosms.

Ecosystem sampling and fish collection

At the end of the mesocosm experiment, we measured ecosystem functions such as gross primary productivity (GPP), ecosystem respiration (ER), turbidity (attenuation of photo-synthetically available light radiation, LKD), sedimentation rate (SED), and bacterial respiration (BR). These five ecosystem functions were combined into a multi-functionality index (Byrnes et al., 2014). We also sampled four main biological ecosystem properties (biomass and diversity of zooplankton, zoobenthos, phytoplankton, and periphyton). For zooplankton, 10L of water was filtered through a 95μm mesh and preserved at -20^{o}C for further identification and analyses. Zoobenthos was collected from the sediment from a plastic box (10.5 x 10.5 cm) pre-installed at the start of the experiment in each tank. Zooplankton and zoobenthos were identified at least to the order level, and the average biomass of each taxon was estimated by applying length-weight regressions (Table-S2). Phytoplankton biomass, periphyton cover, GPP, ER, LKD, SED, and BR were measured as described in Matthews et al. (2016). Two days after sampling the ecosystem, all surviving fish were collected, euthanized with an overdose of MS222, and immediately scanned for *Gyrodactylus* spp., *I. multifiliis*, *Diplostomum* spp. and *Apatemon* spp. under a dissection scope (Leica MZ6). Infections by *Schistocephalus solidus* where neither observed in the laboratory nor the mesocosm experiments. For each fish, we measured standard body length (mm) and wet body mass (g) to obtain an estimate of individual body condition (Peig et al., 2009).

Data Analyses

Parasite load and body condition

We tested for differences in parasite load and composition using generalized multivariate models (Wang et al., 2012), with four focal parasites (e.g. *Apatemon* spp., *Diplostomum* spp., *Gyrodactylus* spp., and *I. multifiliis*), and the factorial treatments and experimental block as fixed effects. For body condition, we used linear mixed effect models (LMM) with treatments and the parasite species with the strongest negative effect on body condition (see Figure-S1 in Appendix S1) as covariates, and tank nested within block as a random effect. Fish mortality (number of dead fish/introduced fish per tank) was modeled using generalized linear models (GLMM) with a

binomial-logit error distribution.

Direct and indirect ecosystem effects

We tested the effects of fish lineage, density, and parasite load on trophic cascades and ecosystem functioning in three complementary ways. First, we tested the effects of the treatment combinations on the biomass and structure of prey communities (e.g., zooplankton and zoobenthos) with multivariate-based models, and on primary producers (e.g., phytoplankton biomass and periphyton cover) and ecosystem multi-functionality index (EMF) with LMMs using fish lineage x density x parasite load as fixed effects and block as a random effect. In the multivariate analyses, we used block as a fixed effect to control for the effects of block. EMF was calculated by standardizing all ecosystem functions (GPP, ER, BR, SR, and LKD) using *z*-scores, and then averaging them following Byrnes et al. (2014). We used an average index to measure ecosystem functioning because we are interested in the simultaneous effect of the factorial treatments on multiple ecosystem functions, so that high values of EMF will represent high levels of ecosystem productivity (Manning et al., 2018). Overall, this analysis approach tests for treatment effects on individual and composite (e.g. EMF index) metrics, but not for covariation between metrics that would provide evidence for trophic cascades. In our experiment, evidence for trophic cascades would exist if increasing fish density both increases primary producer biomass (i.e. benthic or pelagic algae), in addition to either decreasing consumer biomass or altering consumer species composition (i.e. zooplankton or zoobenthos).

Second, in order to test for the occurrence of trophic cascades, we used natural log-response ratios (LRR) of changing fish density using a Bayesian framework by modelling the effects of lineage x density x parasite load then calculating the posterior distribution of predicted values for each treatment combination (see supplementary methods in Appendix 1) . The effects of changing stickleback density, were then estimated as posterior probability density of $ln(V_{HD}/V_{LD})$ where V_{HD} and V_{LD} denote, respectively, the value of a variable (V) at high and low- fish density. For example, the effects of stickleback density on algae were measured as $ln(A_{HD}/A_{LD})$. In this case, we consider evidence for a trophic cascade when the posterior probability of algae LRR is significantly positive and the posterior probability of grazer LRR of

either grazer biomass or composition is significantly negative. Significance is determined by whether the 95% credible intervals (CI) overlap with zero or not for both axes (Shurin et al., 2002). Using the posterior distribution from the Bayesian model allows us to estimate the posterior probability of a trophic cascade.

Third, in order to infer causal relationships between the treatments, grazer communities, primary producers, and the EMF index, we built a piecewise structural equation model (SEM, Lefcheck, 2016) (Figure-S2 in Appendix S1). In the causal network approach, we used fish density and parasite load as exogenous variables, grazer community biomass and composition as intermediates (i.e. between fish and primary producers), and EMF as the end point. We consider evidence of trophic cascades from the SEM model when adjacent trophic modules (e.g. zooplankton and algae) are connected by significant negative effects. Piecewise SEM is a particularly useful method to test for trophic cascades because it can (i) piece together multiple (generalized) linear models into a single causal network to estimate significant indirect effects, (ii) control for the effects of random structures, and (iii) compare nested models, while correcting for small sample size (Lefcheck, 2016). The overall fit of a piecewise SEM is tested using the Shipley's test of d-separation (Shipley, 2009), which validates missing paths, which are not included in the model but that can improve its fit. To reject or accept a SEM model, the combined probabilities of all the paths are compared with a chi-square distribution with $2k$ degrees of freedom (i.e. number of independent claims). If the resulting $C - Fisher's$ value is unlikely to have occurred by chance ($P < 0.05$) then the SEM model is rejected because it does not have a good fit to the data (Shipley, 2009; Lefcheck, 2016). Additionally, we tested whether fish lineage had contrasting effects by comparing a multigroup-SEM with an overall model (Shipley, 2002). With this approach, we could test for the lineage effects both on the entire model and on each causal path independently (Shipley, 2002).

All statistical analyses were performed in R-v3.2.3 (R Core Team, 2015). To test the multivariate effects of the treatments on the structure of the parasites community, zooplankton community, zoobenthos community, and ecosystem function, we used multivariate models with the functions $manlyglm$ or $manylm$ from the R-package *mvabund* (Wang et al., 2012). We calculated statistical significance of each treatment with a multivariate likelihood-ratio test (MLRT) by re-sampling the data 999 times. The Bayesian models were performed using the R package

rethinking (McElreath, 2016) in STAN (Gelman et al., 2015). Models were sampled from four Hamilton Monte Carlo (HMC) chains and 4000 interactions. We used with uninformative priors e.g. $dnormal(0, 100)$ for all the regression prameters, except for the model variances ($dcauchy(0, 1)$)and verified that all four chains converged using the estimated potential scale reduction statistic ($\hat{R}$) (McElreath, 2016).

Results

Parasite community

In the mesocosm experiment, the medication significantly reduced parasite load, which, as in the laboratory experiment, was mainly driven by a reduction of *Gyrodactylus* spp. These effects were stronger for the Constance than the Geneva fish (Fig. 2b). *Apatemon* spp. parasites were more common in Geneva fish, while *I. multifiliis* infections were more common on Constance fish. Fish density also had significant effects on the parasite community, mostly by increasing the densities of *I. multifiliis* and *Gyrodactylus* spp. (Figure-S3 in Appendix S1).

We found that *Gyrodactylus* spp. (r= -0.2, P$<$ 0.05) and *I. multifiliis* (r= -0.15, P$<$ 0.05) had the strongest negative correlation with fish body condition (Figure-S2a in Appendix S1). Therefore, we tested the combined effects of the experimental treatments and the presence and absence of *Gyrodactylus* spp. on body condition. Body condition was lower in Constance fish (LMM, $F_{1,28.402}$= 31.286, P $<$ 0.001), at high fish densities (LMM, $F_{1,46.93}$= 65.290, P $<$ 0.001), and when infected with *Gyrodactylus* spp. (LMM, $F_{1,160.179}$= 4.393, P= 0.025). Constance fish also had higher levels of mortality (GLMM, $X^2_{2,40}$= 10.948, P= 0.01; Mortality: Constance = 28.32% and Geneva: 11.30%). However, since dead fish were removed and replaced, this differential mortality did not lead to sustained density and fish biomass differences between treatments. Fish mortality was not significantly influenced by the disinfection treatment (GLMM, $X^2_{2,40}$= 2.9211, P= 0.232). Overall, the disinfection treatment successfully reduced parasite load and changed the structure of the parasite community, and, as expected, this was associated with higher condition of stickleback in the $-I$ treatment.

Direct and indirect ecosystem effects

At the end of the mesocosm experiment, the presence of stickleback strongly reduced zooplankton biomass, increased zoobenthos biomass, and altered the structure of the zoobenthos community (Fig. 3a,b,d). Within the factorial treatments, zooplankton biomass was marginally affected by the interaction between fish density and parasite load (Fig. 3a), and zoobenthos biomass was marginally affected by fish density (Fig. 3c). Zooplankton community structure, however, was strongly affected by the interaction of lineage, density, and parasite load treatment (Fig. 3b and Table-S3). Specifically, at high density, $-I$ fish had stronger negative effects on copepods ($F_{1,28}$= 8.399, $P < 0.01$); and Geneva fish had stronger positive effects on predatory mites in high density tanks with $-I$ fish (*Acari*, lineage x density x parasite load: $F_{1,28}$= 7.1700, P = 0.012; Figure-S4 and Table-S4 in Appendix S1). Unlike the zooplankton community, variation in the zoobenthos community structure and total biomass did not vary among the lineage, density, and parasite load treatment combinations (Fig. 3b and d). Furthermore, we found that copepods and mayfly larvae (*Caenis*) had the strongest negative correlation with phytoplankton (r=-0.44, $P< 0.05$) and periphyton (r=-0.19, $P<0.05$; see Figure-S2b and c in Appendix S1). Overall, increasing stickleback density increased ecosystem productivity (Fig. 3e-j). At high fish density, mesocosms had more phytoplankton, less periphyton, higher levels of GPP, ER, and EMF than low density tanks (Table-S4 in Appendix 1). We also found that mesocosms with Constance fish had higher levels of ER, LKD, and EMF (Table-S4 in Appendix 1).

We tested for trophic cascades mediated by either changes in grazer biomass (DMIE) or community composition, which could ultimately impact grazer feeding rates and/or be the results of changes in fish behavior (e.g., TMIE). In both cases, there was stronger evidence for a pelagic trophic cascade than for a benthic trophic cascade when increasing fish density (Table 1, Fig. 4). The Bayesian LRR (Fig. 4) and the structural equation model (SEM, Fig. 5a) both revealed that increasing stickleback density strongly impacted zooplankton community composition (MDS1), and this was associated associated with higher phytoplankton biomass and EMF (path: HD $\rightarrow$ Zoo MDS1 $\rightarrow$ Phytoplankton $\rightarrow$ EMF, Fig. 5).

The Bayesian LRR additionally revealed that the probability of trophic cascades was very

similar for both lineages (Table 1). Consistent with this, we found that Constance and Geneva stickleback did not differentially affect the overall structure of the SEM model (i.e. a multi-group SEM was not supported: $X^2_{1,36}$= 49.461, P = 0.066). However, in the overall SEM, we did find lineage-specific differences in parameter estimates relating to pathways underlying trophic cascades (Fig. 5b). These pathways include both the effect of fish density on zooplankton community structure (Fig. 5b), which was 21.6% stronger for Geneva fish ($X^2_{1,95}$=36.139, P $<$ 0.01) and the effect of zooplankton community structure on algal biomass (Fig. 5b), which was 23.2% stronger for Constance stickleback ($X^2_{1,95}$=8.93, P $<$ 0.01, see Table-S7).

Overall, reducing parasite load increased the probability of detecting pelagic trophic cascades by more than 30%, especially for cascading effects mediated by changes in the zooplankton community structure (Table 1 and Fig. 4). This outcome is also consistent with a non-bayesian LRR analysis (Figure-S6 in Appendix 1), and the SEM modelling (Fig. 5a). Whereas the overall effects of a high fish density led to a large shift in zooplankton composition (red arrow with coefficient -0.74), a high density of fish with natural parasite infections levels ($+I$) showed a much weaker impact on zooplankton composition (black arrow with coefficient 0.64 counteracts these negative effects). The weaker effects of fish with natural parasite loads on zooplankton translated into weaker indirect effects on phytoplankton biomass (path: HD:+I $\rightarrow$ Zoo MDS1 $\rightarrow$ Phytoplankton, Fig. 5a).

Discussion

Overall, our experiment tests how parasites can influence the top-down effects of predators on trophic cascades and ecosystem functioning. Specifically, we found that increasing stickleback density caused trophic cascades in the pelagic but not the benthic food chain (Fig. 4). Variation in periphyton cover was also affected by fish density but we found no evidence linking it to changes in zoobenthos biomass or composition (Fig. 5). Pelagic trophic cascades occurred due to effects of stickleback density and parasite load on zooplankton community composition rather than zooplankton biomass (Table 1). Evidence of trophic cascades was stronger when parasite load of fish was reduced (Fig. 4, 5a, Table 1) and for the population with the more pelagic phenotype (i.e. Lake Constance: Fig. 5b). The index of ecosystem multi-functionality, showed stronger support

for lineage and density effects than for parasite effects (Fig. 5, Figure-S5 in Appendix 1), but a large portion of the variation (86%) in EMF remains unexplained in the SEM model.

Although parasites are well known to affect the physiology and behavior of hosts (Barber and Dingemanse, 2010), little is known about the reach of their effects across multiple trophic levels (Wood and Johnson, 2015). Parasites directly influence zooplankton community dynamics (Duffy, 2007), zoobenthos (Mouritsen and Haun, 2008) and primary producers (Ibelings et al., 2004); and some parasites with complex life cycles can even affect multiple trophic levels at different stages of their life cycle (Lafferty and Kuris, 2012). Here, we found that reductions of parasite load (of multiple parasite species), increase likelihood of stickleback-mediated trophic cascades in the pelagic environment (Fig. 5a). Parasites such as *Diplostomum* spp. and *Apatemon* spp., which can impair fish vision, might have influenced the experimental fish, but we did not find strong evidence of their deleterious effects. Instead, our results suggest that *Gyrodactylus* spp. and *I. multifiliis* reduced the condition of stickleback (Eizaguirre et al., 2009, 2012), potentially altering foraging performance and behavior and leading to cascading effects on primary producers (Anaya-Rojas et al., 2016; Brunner et al., 2017).

While many traits of prey (e.g. induced defences, anti-predator avoidance behaviors) are well known to play important roles in trophic cascades (Schmitz et al., 2004), much less is known about the importance of predator traits (Abrams, 2007; Lima, 2002), particularly those underlying foraging performance. Predator traits such as foraging activity (Abrams, 1982) and diet choice (Ma et al., 2003) could be particularly susceptible to parasite infections due to behavioral and physiological trade-offs (Hatcher and Dunn, 2011). This is consistent with our observations that the reduction of parasite load affected prey community composition but not overall biomass, and these changes led to an increased on algal biomass. Such trophic cascades have been previously dubbed cryptic, because they are difficult to detect without careful quantification of changes in community composition (Tessier and Woodruff, 2002). Indeed, it is possible that the positive effect of fish on periphyton biomass (Fig. 5a) might reflect undetected changes either in the species or trait distributions of the benthic community, or in the physical and chemical environment (e.g. habitat modification, nutrient cycling).

Trophic cascades involving changes in community composition can be both common and strong in natural populations (Schmitz, 2006; Tessier and Woodruff, 2002). Factors such as

predator or herbivore foraging efficiency (e.g. numerical dynamics and functional response) and community regulation (e.g. compensatory effects), for instance, can increase the strength of cryptic trophic cascades via changes in consumer conversion efficiency even without changes in the total community biomass (Borer et al., 2005; Pace, 1984). In our experiment, it is likely that stickleback at high densities reduced large and efficient zooplankton grazers, without reducing the total biomass of zooplankton. It is also possible, that the observed changes in zooplankton community structure influenced the structure of the phytoplankton and microbial community, which has been observed in previous mesocosm experiments (Birtel and Matthews, 2016). Additional quantification of these communities might have improved the explanatory power of the pathways identified by the SEM analysis, and helped identify additional pathways linking fish effects with changes in ecosystem functioning (Fig. 5b).

Overall, our analyses suggest that stickleback from the Lake Constance and Lake Geneva indirectly modified mesocosm ecosystems through similar ecological mechanisms, specifically via pelagic trophic cascades mediated by changes in zooplankton community structure. However, in the more detailed analysis of SEM pathways, we found some evidence that the two lineages had differential effects on pelagic trophic cascades and on periphyton (Fig. 5b). For example, differences in path coefficients between lineages suggest that fish from Constance caused stronger pelagic trophic cascades. Specifically, the net effect of Constance stickleback density on phytoplankton biomass was greater (i.e. more positive), relative to their effect on zoooplankton composition (Fig. 5b). It is unclear why fish from both lineages had strong density effects on copepods, but this only translated into a significant change in phytoplankton biomass in the Constance treatment (Table-S7 in Appendix S1).

Previous work suggests that Lake Geneva sticklebacks are more adapted to foraging in benthic environments, whereas Lake Constance stickleback are more adapted to pelagic environments (Lucek et al., 2013, 2014; Best et al., 2017). For instance, stickleback from Lake Constance have a more pelagic phenotype (Lucek et al., 2013) and have a higher feeding efficiency on plankton (Best et al., 2017) than stickleback from Lake Geneva. One possibility is that Constance fish had a stronger impact on phytoplankton, because they fed more selectively on more efficient grazer, but a remaining challenge is to identify which of the traits that differ between lineages are responsible for their differential community and ecosystem effects (Best

et al., 2017). Lineage differences in the effect on periphyton cover could also suggest a role for non-trophic effects of stickleback on ecosystem functioning (Atkinson et al., 2017), suggesting further work is needed on mechanisms such as habitat modification and nutrient cycling (Best et al., 2017; Leal et al., 2017).

There are some limitations to our experiment that prevent us from making stronger and more general conclusions about the effects of parasite load and evolutionary lineage of stickleback on aquatic ecosystems. Firstly, since the parasite manipulations were performed on wild-caught fish, a combination of phenotypic plasticity and genetic differences are likely to explain some variation in the effects of fish on grazer communities and ecosystem functioning (Lundsgaard-Hansen et al., 2014; Brunner et al., 2017). However, our results show that independently of the origin of the effect, stickleback parasites can affect trophic cascades. Secondly, replacing fish that died during the experiment with fish from the lab was essential to maintain the density contrast throughout the experiment, but it also meant that fish in higher condition from the lab were substituted into the experiment on multiple occasions. However, since parasites such as *Gyrodactylus* spp. and *I. multifiliis* can be easily transmitted among fish, it is likely that replacement fish would have been exposed and infected, maintaining the indirect effects of parasites on the system (Eizaguirre et al., 2009).

In this study, we tested how parasites at the top of food chains can indirectly influence grazer communities and ecosystem functioning. Such cascading effects are likely to be influenced by co-evolutionary interactions between host and parasites that vary with the environmental context (Thompson, 2013; Brunner et al., 2017). Overall, our results suggest that parasites can affect ecosystems via a combination of both DMIE and TMIE, mediated by host condition and behavior of the predator. This highlights the importance of adopting both ecological and (co)evolutionary perspectives for studying the effects of species interactions on community structure and ecosystem processes.

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Table 1: Posterior probability (%) of detecting density mediated trophic cascades given stickleback *Lineage* and *Parasite load*. Pelagic trophic cascades are more likely in mesocosms with $-I$ fish ($>$ 83%). The probability of detecting benthic trophic cascades was $<$ 50% for any treatment.

	Pelagic trophic cascades (%)					
	Grazer's biomass			**Grazer's structure**		
	Constance	*Geneva*	*Parasite load*	*Constance*	*Geneva*	*Parasite load*
+I	50.05	50.30	50.04	51.22	50.75	51.79
-I	75.18	83.54	83.23	99.13	96.91	99.61
Lineage	54.40	50.67		52.09	51.6	
	Benthic trophic cascade (%)					
+I	48.93	49.19	48.93	49.56	43.44	49.59
-I	7.88	30.25	10.88	29.52	23.52	21.08
Lineage	48.47	48.98		49.325	49.33	

List of figures

1. Examples of trophic cascades in food webs with three trophic levels (a and b) and four trophic levels (c). Diagrams **a** and **c** represent food chains in which an algae population or community (A) is eaten by a grazer population or community (G), which is eaten by a predator population (P). Diagram **c** represents a food chain in which the predator population (P) is infected by a parasite (I). Grey circles on the right-hand side denote trait values or trait distributions influencing the species interaction (G_t, a predator avoidance trait; or P_t, a predator foraging trait that is influenced by a parasite infection). Black and gray arrows represent direct density and trait effects. In food chain **a** there is a significant trophic cascade through DMIE but not DMIE on the algae population, unlike food chains **b** and **c**. In food chain **c**, parasite infections have significant trophic cascading effects on grazer and algae population.

2. Experimental design **(a)** and the effects on *Gyrodactylus* spp. on body condition **(b)** and **(c)**. In **(c)**, G+ and G- stand for the presence and absence *Gyrodactylus* spp.. Symbols show the experimental group means +/-SE and numbers over symbols indicate the number of individuals in each category (see Table-S3 in Appendix 1 for more details in all parasites).

3. Effects of fish presence (F), lineage (Constance and Geneva), density (High and Low), and infection (*+I* and *-I*) on ecosystem properties and functions. Panels (c) and (d) show the effects on the first multidimensional axis of the zooplankton and zoobenthos communities, respectively (see Figure-S2 in Appendix S1). Symbols represent mean values and bars standard errors (s.e.) (see Tables S2 and S3 for more details).

4. Relationship between the direct effects ($x - axis$) of stickleback on grazers (pelagic: a and b; benthic: c and d) and the indirect effects ($Y - axis$) on algae (pelagic: a and b; benthic: c and d). Symbols show the net effect of changes in fish density given stickleback lineage and parasite load. There is evidence of significant trophic cascading effects when the point estimate of the effect (mode from the posterior distribution) magnitudes are in the gray area and the 95% credible interval are not overlapping with the zero line (see Shurin et al., 2002). Significant trophic cascades are highlighted with an (*). For more details on the Bayesian

models see Table-S6 in Appendix 1.

5. Results of the SEM model. Diagram (a) shows the best overall model, and diagram (b) shows the same model but highlighting the lineage differences in path coefficients. Here, We only present significant path coefficients representing positive (black) and negative (red) effects (but see Table-S5 and S7 for more details). Trophic modules involved in significant trophic interactions are shaded. Numbers indicate standardized effect size (-1 to 1) of the path coefficients. The bottom right corner shows the model fitting the data for the best model ($P > 0.05$). As exogenous variables we modeled the effects of high fish density (HD) and natural infection levels ($+I$).

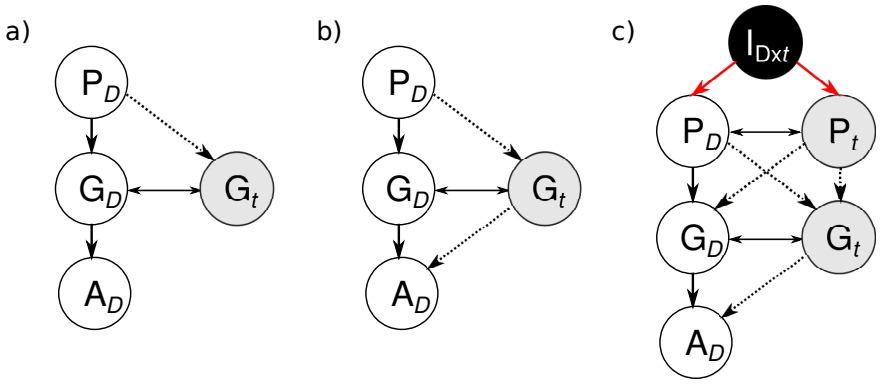


Figure 1:

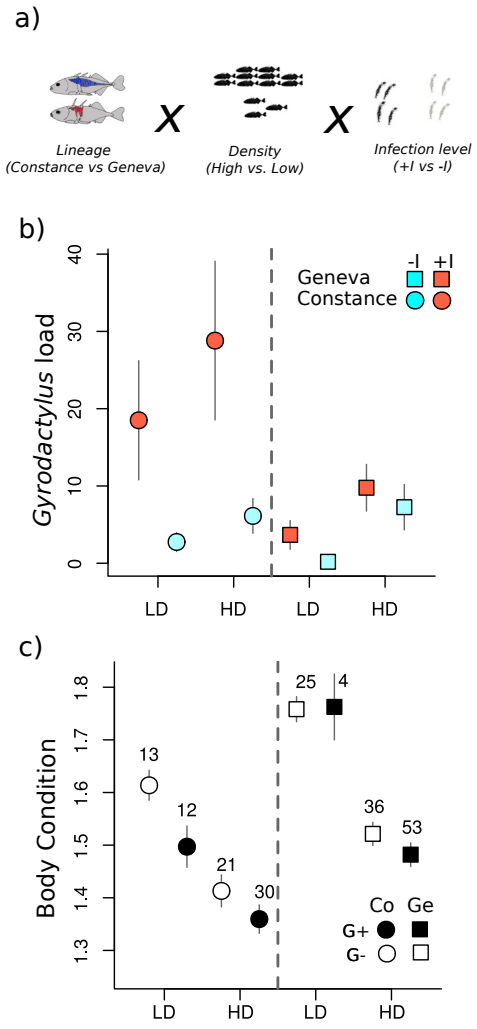

a)
Lineage
(Constance vs Geneva)
X
Density
(High vs. Low)
X
Infection level
(+I vs -I)
b)
Gyrodactylus load
0
10
20
30
40
-I
+I
Geneva
Constance
LD
HD
LD
HD
c)
Body Condition
1.3
1.4
1.5
1.6
1.7
1.8
13
12
21
30
25
4
36
53
Co
Ge
G+
G-
LD
HD
LD
HD

Figure 2:

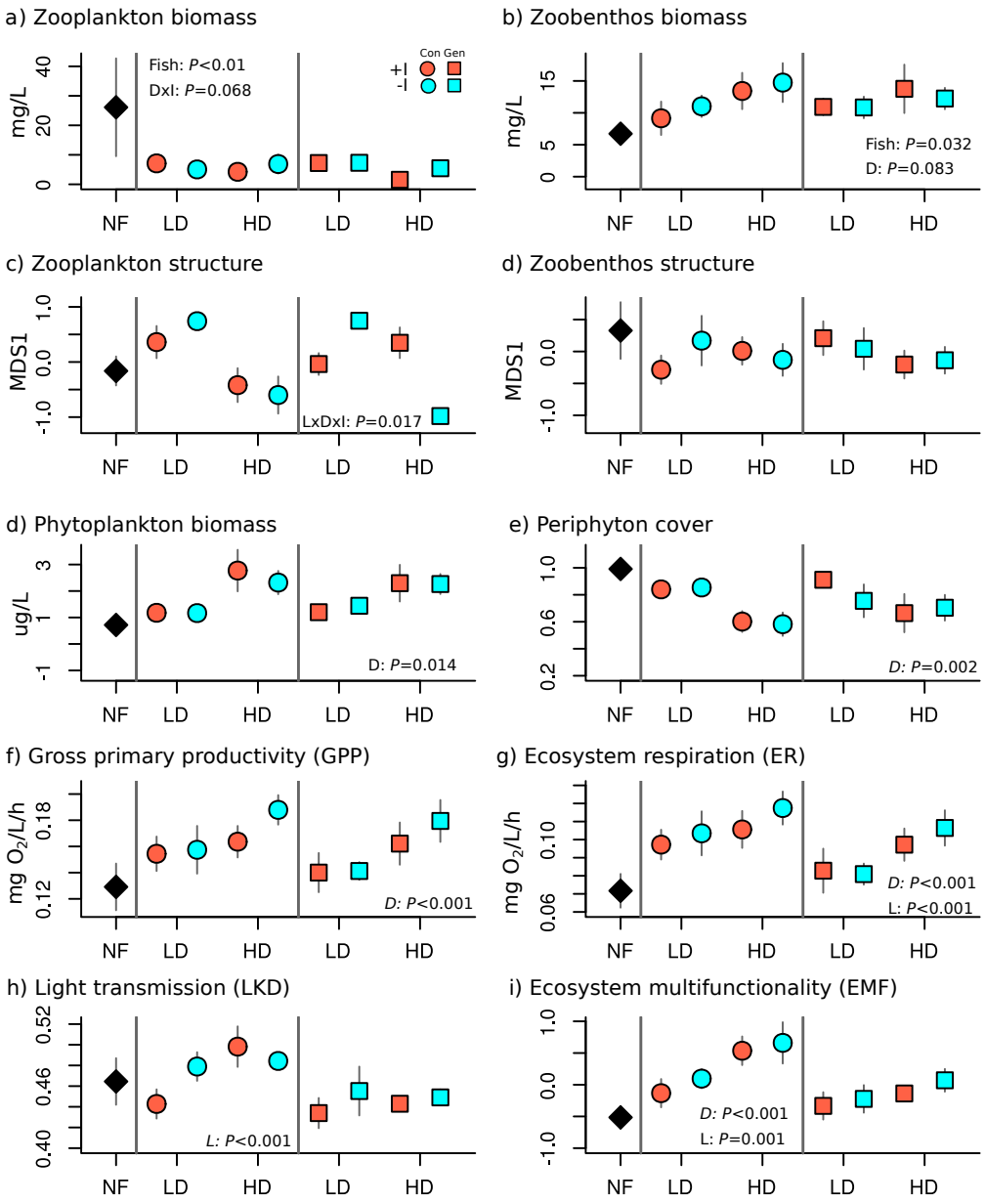

a) Zooplankton biomass
mg/L
0
20
40
Fish: P<0.01
DxI: P=0.068
Con Gen
+I
-I
NF
LD
HD
LD
HD
b) Zoobenthos biomass
mg/L
0
5
15
Fish: P=0.032
D: P=0.083
c) Zooplankton structure
MDS1
-1.0
0.0
1.0
LxDxI: P=0.017
d) Zoobenthos structure
MDS1
-1.0
0.0
d) Phytoplankton biomass
ug/L
-1
1
3
D: P=0.014
e) Periphyton cover
0.2
0.6
1.0
D: P=0.002
f) Gross primary productivity (GPP)
mg O2/L/h
0.12
0.18
D: P<0.001
g) Ecosystem respiration (ER)
mg O2/L/h
0.06
0.10
D: P<0.001
L: P<0.001
h) Light transmission (LKD)
0.40
0.46
0.52
L: P<0.001
i) Ecosystem multifunctionality (EMF)
-1.0
0.0
1.0
D: P<0.001
L: P=0.001

Figure 3:

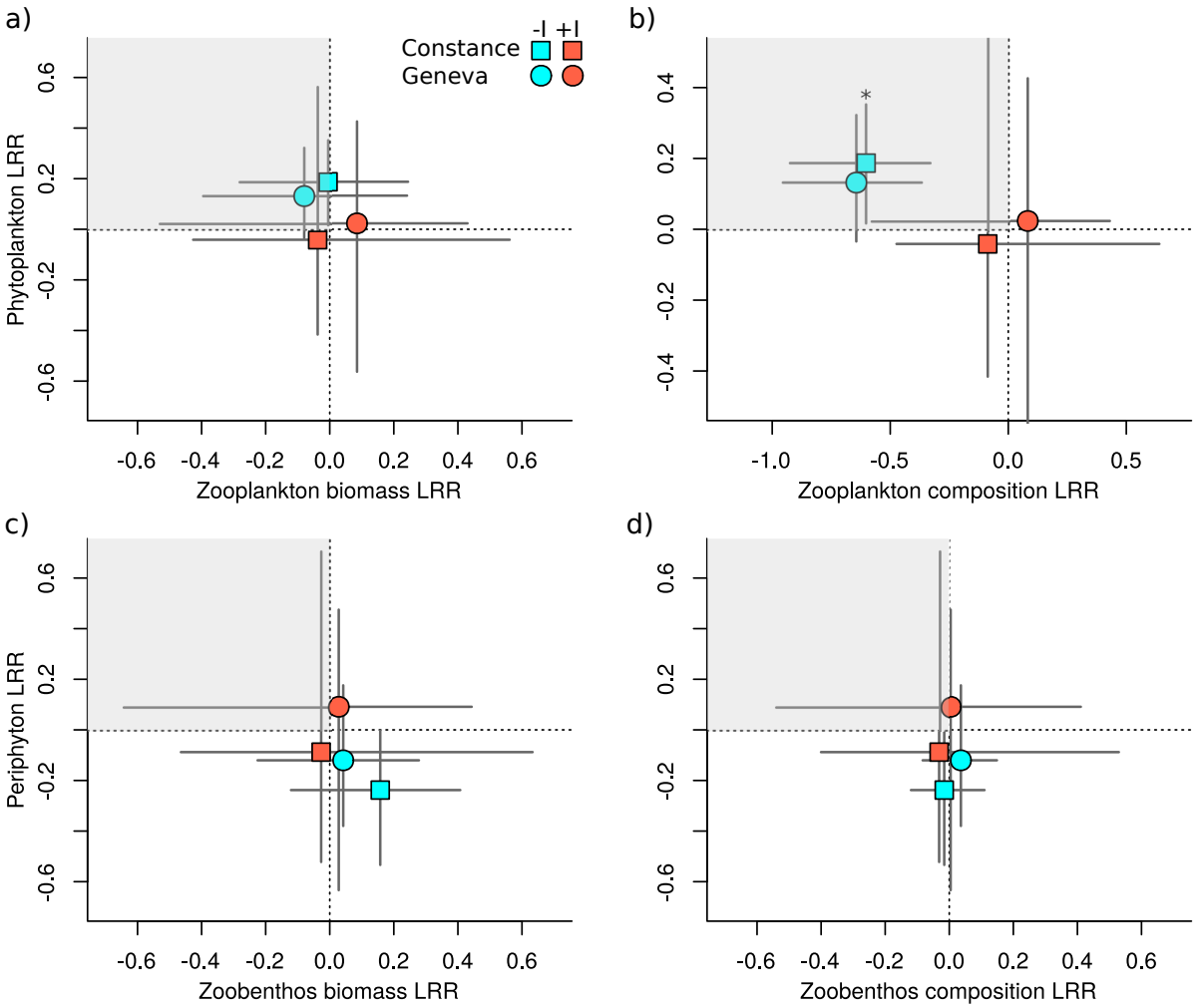
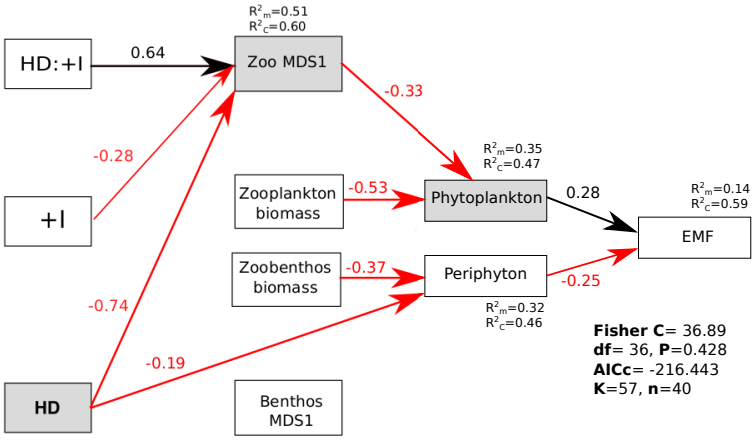

a)
b)
c)
d)
-I +I
Constance
Geneva
Phytoplankton LRR
Periphyton LRR
Zooplankton biomass LRR
Zooplankton composition LRR
Zoobenthos biomass LRR
Zoobenthos composition LRR
*

Figure 4:

a) Overall model



b) Overall model showing lineage differences

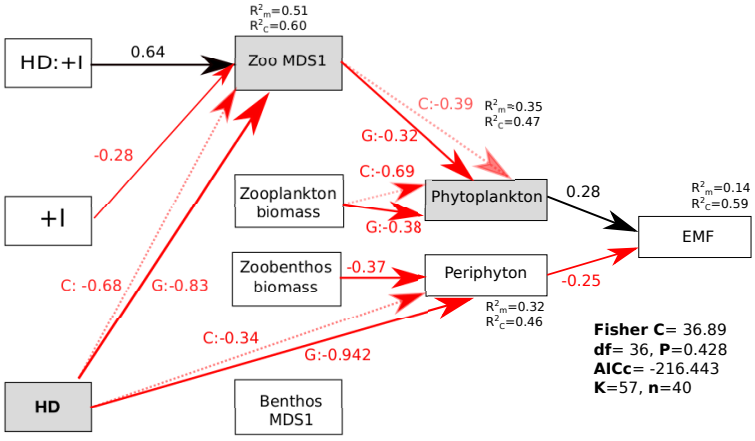


Figure 5: